
Generation of Genetically Modified Organotypic Skin Cultures Using Devitalized Human Dermis.

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Public Summary:

Organotypic cultures allow the reconstitution of a 3D environment critical for cell-cell contact and cell-matrix interactions which mimics the function and physiology of their in vivo tissue counterparts. This is exemplified by organotypic skin cultures which faithfully recapitulates the epidermal differentiation and stratification program. Primary human epidermal keratinocytes are genetically manipulable through retroviruses where genes can be easily overexpressed or knocked down. These genetically modified keratinocytes can then be used to regenerate human epidermis in organotypic skin cultures providing a powerful model to study genetic pathways impacting epidermal growth, differentiation, and disease progression. The protocols presented here describe methods to prepare devitalized human dermis as well as to genetically manipulate primary human keratinocytes in order to generate organotypic skin cultures. Regenerated human skin can be used in downstream applications such as gene expression profiling, immunostaining, and chromatin immunoprecipitations followed by high throughput sequencing. Thus, generation of these genetically modified organotypic skin cultures will allow the determination of genes that are critical for maintaining skin homeostasis.

Scientific Abstract:

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